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Summary

Cancer is characterized by uncontrolled cell growth and is caused by alterations in genes that regulate the cell cycle, apoptosis and communication between cells. These alterations are essentially due to misrepaired DNA damage, which may result from DNA replication errors as well as from chemically reactive agents produced by both endogenous and exogenous mechanisms. Yet, the spontaneous mutation rate in healthy individuals is unable to explain the life-time cancer risk. This is because cells are equipped with sophisticated molecular systems that are highly efficient in maintaining the integrity of the genome and keeping the mutation rate low. Failure of these systems to protect the genome increases the mutational load and increases the chance for growth-controlling genes to become dysregulated. That is precisely the case in individuals who suffer from genetic instability disorders, of which Fanconi anemia (FA) is a well-known example.

FA is a rare recessively inherited disorder characterized by diverse clinical symptoms, including organ malformations, bone marrow failure and increased cancer risk. To date, 13 genes have been identified that, when mutated, can cause FA. Among these genes, the breast/ovarian cancer susceptibility gene, *BRCA2*, has been found defective in a subgroup of FA patients. The proteins encoded by the FA genes appear to function in a distinct biochemical pathway, the 'FA/BRCA pathway', which acts to stabilize the genome. A defect in this pathway is not lethal, but has profound consequences, both at the cellular and clinical levels. Cells with an FA/BRCA pathway defect exhibit an extremely high sensitivity to DNA cross-linking agents, such as the chemotherapeutic agents cisplatin and mitomycin C (MMC).

The purpose of this thesis was two-fold. First, to expand our knowledge of the pathway by searching for novel genes that participate in the pathway (Chapters 2-4). Second, to explore the possible occurrence and significance of FA/BRCA pathway defects in sporadic cancer (Chapters 5-8).

In **Chapter 2** a comprehensive genetic subtyping approach for FA is presented that is primarily based on mutation screening, supplemented by protein expression analysis and by functional assays to test for pathogenicity of unclassified variants. A total of 80 unselected FA patients were analyzed, of which 73 could be successfully subtyped. Ninety-two distinct mutations were detected, of which 56 were novel. All known genetic subtypes were represented, except D2, J, L, and M. Four patients

could not be assigned to any of the known subtypes; these patients may represent novel subtypes. One of these patients had a defect downstream of the FANCD2 monoubiquitination, whereas the remaining 3 were defective upstream of this step. The conclusion from this study was that direct mutation screening, in combination with functional tests, allows a molecular diagnosis of FA in the vast majority of patients. The patients that were unclassifiable by this approach likely represent novel genetic subtypes for which the underlying gene defect remains to be identified.

Chapter 3 reports that for one of these patients the defect could be traced to a gene that was known to encode PALB2, a protein that is essential for the stabilization and localization of BRCA2. This subtype, which was named FA-N, is characterized by relatively severe clinical symptoms and extreme susceptibility to childhood cancers, similar to the FA-D1 subtype (mutated in *BRCA2*). Furthermore, as reported by others, individuals who carry a heterozygous mutation in this gene appear to have an increased risk to develop breast cancer. In **Chapter 4**, the occurrence of large *PALB2* deletions, such as identified in the FA-N patient described in **Chapter 3**, was assessed in a large cohort (734) of familial breast cancer (FBC) patients but did not reveal the involvement of such aberrations in BC risk.

The extreme proneness of FA patients to develop malignancies such as acute myeloid leukemia (AML) and squamous cell carcinoma of the head and neck region, suggested the involvement of the FA/BRCA deficiency as an underlying mechanism in a proportion of these 'sporadic' malignancies in the general population. In **Chapter 5**, a functional disruption of the FA/BRCA pathway in a leukemic cell line was demonstrated to occur through promoter methylation of the *FANCF* gene, which codes for an essential component of the pathway. In contrast to gene-disrupting mutations, promoter methylation is another mechanism by which genes can be inactivated and is frequently observed for other genes in various types of malignancies. These *FANCF* deficient cells were hypersensitive to MMC, while reintroduction of the active *FANCF* gene into these cells restored resistance to MMC. Promoter methylation of two other FA genes, *FANCC* and *FANCL*, in cells from sporadic leukemia patients (AML and ALL) is described in **Chapter 6**. In these cases the methylation was also correlated with hypersensitivity to MMC. To investigate the possible predictive value of *FANCF* promoter methylation in head-and-neck squamous cell carcinoma patients towards treatment strategies containing cisplatin, a panel of 22 patients of whom 11 responded favorably to the treatment were assessed

for *FANCF* methylation in **Chapter 7**. In contrast to previous reports, promoter methylation was absent in all patients, which might partially be explained by the used detection method that is prone to produce false-positive results.

In an attempt to assess the involvement of the FA/BRCA pathway defects in sporadic cancer, 25 cell lines derived from tumors that occur frequently in FA patients were evaluated for their FA/BRCA pathway status (**Chapter 8**). Interestingly, as many as 65% of the cell lines demonstrated FA-like behavior in terms of sensitivity to MMC, suggesting an abrogated FA/BRCA pathway. All cell lines appeared to be able to monoubiquitinate FANCD2, suggesting a defect downstream of this step. However, only in a small portion of the MMC-sensitive cell lines could this phenotype be correlated with molecular defects: BRCA2 protein was undetectable in two cell lines, while BRCA1 appeared to be absent in 3 cell lines. The molecular basis for these aberrations was unclear, since we were unable to find sequence alterations in these genes that could explain the protein deficiencies. However, inactivation of the FA/BRCA pathway in the remaining “FA-like cell lines” could not be attributed to any of the known FA proteins, suggesting the involvement of (a) novel FA protein(s). Further analysis of these cell lines may result in the identification of new important player(s) in the FA/BRCA pathway. These possible new FA genes may turn out to be frequent targets that are inactivated in the oncogenesis process and may thus also serve as predictors for cancer treatment response.

In conclusion, the work presented in this thesis has contributed to the identification of a new breast cancer susceptibility gene, *FANCN*. Furthermore, evidence for novel FA genes yet to be identified has been presented. Identifying these genes will enhance our understanding of the FA/BRCA pathway. Given that inactivation of the FA/BRCA pathway renders cells hypersensitive to specific chemotherapeutic agents, identification of malignancies with such a defect may pinpoint patients that could benefit from adapted treatment strategies that exploit the Achilles heel of such malignancies: hypersensitivity to cross-linking agents.